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FOUL BROOD

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Neither a borrower nor a lender be
Quoth Shakespere. lest both loan be
lost and friend.
If then I break the rule to pleasure thee
Keep not I pray the book I only lend.
Hiatus Valde Defleti.
Ex
Col. H. J. O. Walker. Lee
Ford
Budleigh Salterton.

ONTARIO AGRICULTURAL COLLEGE

BULLETIN 112.

FOUL BROOD OF BEES

BY

F. C. HARRISON, B.S.A., PROFESSOR OF BACTERIOLOGY.

ONTARIO DEPARTMENT OF AGRICULTURE,
TORONTO, ONT., DECEMBER, 1900.

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1900

ONTARIO DEPARTMENT OF AGRICULTURE.

BULLETIN 112. Toronto, December, 1900.

AGRICULTURAL COLLEGE AND EXPERIMENTAL FARM.

FOUL BROOD OF BEES.

BY F. C. HARRISON, PROFESSOR OF BACTERIOLOGY.

HISTORICAL RESUMÉ.

In all probability the first definite reference to foul brood is by Aristotle (1), who mentions an inertness which seizes the bees, and causes a bad smell in the hive. He also suggests that bees are liable to become diseased when the flowers on which they work are attacked by blight. Bee dysentery causes a bad odor as well as foul brood; but in the former disease the spotting and consequent smell are usually outside the hive.

Columella (2) mentions a bee pestilence and an annual distemper which seizes the bees in spring. Pliny (3) writes of a disease of bees, but as he uses the same term as Aristotle he has probably copied it from the latter author.

Schirach (4) in 1769 was the first writer to name the disease "Foul Brood." He says that "it is dangerous, and a most destructive disorder to the bees, a genuine plague when the complaint has reached a certain stage. The cause can be attributed to two sources—1. The putrid (or tainted) food with which the bees feed the larvæ for lack of having better. 2. By the mistake of the queen bee, in misplacing the larvæ in their cells, head upside down. In this position the young bee, unable to get out of its prison, dies and rots away." Further, Schirach clearly distinguishes between foul brood and chilled brood, and mentions the fact, that putrefaction follows the death of the brood from frost, but in this case "it is only an accident and not a disease."

The remedy Schirach recommended was to remove all diseased combs from the infected hives and to keep the bees fasting for two days, after which they are furnished with other cakes of wax, and a suitable remedy given, "as a little hot water mixed with honey, nutmeg and saffron, or a syrup composed of equal parts of sugar and wine, seasoned with nutmeg." Thus, as Cowan (5) remarks "we had given us nearly 130 years ago, a method of cure almost identical with what is by some claimed as new to-day."

Tessier (6) about the same time as Schirach, says, that when the larvæ die in their cells it causes an infection in the hive which makes the bees sick. It is then necessary to drive away or sometimes move the bees from the hive, and to take care to fumigate the infected hive if it is going to be used again. It is necessary, in order to avoid the same inconvenience, to take out the parts of the comb that may be moulded by reason of the dampness. Duchet (7), who wrote on bees in 1771, does not mention any disease that can be certified as foul brood, but he describes bee dysentery.

Della Rocca (8), Vicaire-General of Syra, an island in the Levant, relates with much detail the history of an epidemic of foul brood, which caused great destruction in the island during the years 1777 to 1780. Della Rocca describes very minutely the symptoms, destruction and mistakes that were made in attempting to combat the disease. "The disease," he says, "manifests its presence by defects in the combs filled with brood, and which only contain a putrid mass; instead of the bee pupæ there is only rottenness in the cells, which, however, being capped always preserve a healthy appearance. If these cells are broken open, a blackish liquid flows out, which spreads the infection through the hive. This disease only manifests itself in cells which contain a nearly mature larva or a capped one. The bees themselves remain in good health, and work with the same activity, but their numbers decrease daily. This disease, however, was not so general in a hive but that a small portion escaped; some new bees emerged, but in too small numbers to supply the daily losses Thus a hive attacked by this scourge will perish from scarcity of population. At first it was not noticed that this disease was epidemic, and the hives emptied by death of the bees, were filled with fresh swarms, and these contracted the same disease and perished. Yet another mistake was made. The remains of the hives that were lost were taken into the streets of the town to expose them to the sun, in order to extract all the wax, and the bees from the neighborhood sucked up the honey, caught the disease, and communicated it to other hives, and all, without exception perished in a short time. The epidemic, having reached the island, spread everywhere and the mortality among the bees was general, either from eating infected honey, or from stopping up the infected combs, or from the bees nourishing their brood on infected honey." Della Rocca criticizes Schirach's statement regarding the misplacement of the larvæ by the queen as a cause of the disease, because "everybody knows that the queen has nothing else to do but deposit eggs. These are then cared for and nourished by the bees, and when the larva is nearly ready to change into the pupa, the bees close the cell, and every position which is given the larva depends on their good pleasure and not on the queen's." Della Rocca himself thinks that "some pestilential blight had without doubt corrupted the quality of the honey and the dust from the anthers," and recommends "burning everything without pity, as there is no other resource when the disease is well established, as the pest is without doubt the most terrible in the natural history of bees."

Neither Wildman (9), Keys (10), Woolridge, Needham (11), Rhein, Reaumur (12), or other authors about the same time (latter end of the 18th century) mention this disease.

Bevan (13) names the disease "Pestilence," and also quotes Schirach's name "Foul Brood," and says regarding it, that the "Pestilence has been attributed to the residence of dead larvæ in the cells, from a careless deposition of ova by the queen . . . it has also been attributed to cold and bad nursing, that is, feeding with unwholesome food."

Nothing further of note appears in bee literature till the year 1860, when Dr. Leuckhart (14) writes that he was formerly of the opinion that foul brood was caused by the same fungus (*Panhistophyton ovatum*) which is noticed in a disease of the silk worm, but now after observation and experiment, is quite certain that the disease is caused by neither vegetable nor animal parasite. He also notes that the term "foul brood" is applied to a number of diseases affecting bees.

Molitor Mühlfeld (15) recognizes two forms, one contagious and the other not contagious, and thinks that the only cause of contagious foul brood is a fly (*Ichneumon apium mellificarium*) which lays its eggs on the young larvae of the bee.

A discovery of note was Preuss's (16) in 1868. He contradicts Mühlfeld's statement about the fly, and states that foul brood cells can be detected by the sunken cap. With a microscope magnifying 600 diameters, he found small dust like bodies with a diameter of $\frac{1}{500}$ m. m. belonging to the genus *Cryptococcus* (Kutzig) and called the specific form *alvearis*, likened it to the fermentation fungus (*Cryptococcus fermentum*) and thought that the last germ gained access to the young bee and changed to *Cryptococcus alvearis*. He notices that many experts lay the cause of the disease to fermenting food but the larvae are easily contaminated by the fermentation fungus which is always present in the air. He also mentions the enormous rapidity with which the fungus multiplies and gives an elaborate calculation of the number that might be found in a cell containing a deceased larva

As might have been expected, Preuss's statement aroused considerable discussion at the meeting of German bee-keepers, a short while after the publication of his paper.

Vogel (17) expressed doubt as to whether *C. alvearis* was the real cause of foul brood or only a consequence of the disease, but on the whole agreed with Preuss.

Wiegand (17) agreed with Preuss's theory, and in giving his experience said that the disease was introduced into his apiary through honey brought from a distance.

Pollman (17) believed that the disease was introduced by feeding honey from Havanna, where, when extracting the honey, both brood and honeycomb were mashed up and the honey pressed out.

Dr. Leuckhart (17) agreed with those who thought the disease due to a fungus, but discredited the supposition that it was the same as the fermentation fungus mentioned by Preuss, and rather thought it was related to the silk worm fungus and that most of the brood diseases ending in death were called "foul brood" while they were really something else. He believed that the fungus was present in the eggs of the queen when laid.

Geilen (17) believed that the disease came from the putrefying remains of animal bodies, upon which the bees alighted.

Mühlfeld (18) again in 1869, presented his former views, and also those of Preuss's and gave directions for maintaining the health of bees. He recommended the boiling of the honey, and a use of carbolic acid in the strength of 1:100 or permanganate of potash 1:300 as disinfectants.

Lambrecht (19) thought that foul brood was caused by the fermentation of the bee bread.

Hallier (19) considered it no specific disease, but thought it was probably produced by different fungi.

Cornallia (20) proved contrary to the above and found a fungus, which he thought developed foul brood. He called it *Cryptococcus alveaxis* and used carbolic acid, potassium permag , and lime water as disinfectants.

Fisher (21) advanced a new foul brood theory in 1871, which somewhat follows the view of Liebeg regarding the silk worm disease and plant diseases. According to this theory, the predisposing cause was insufficient nourishment, especially short stores for winter and spring. Shortage of pollen supply was the next cause. Fisher tried to prove his views by the practical experience of bee keepers and explained that the first result of repeated and continued feeding was an increase in the production of bees; and a consequent disproportion between brood and brood feeders arose, which should be looked upon as another cause of foul brood. The disease, he said, might be lessened or exterminated by applying means to reduce the production of brood, as the removing of the queen and the area which the brood occupied. Foul brood is probably the cause of a quantitative dearth of nourishment and a consequent degeneration of the bees. The appearance of fungous growths was only a secondary matter.

Schonfeld (22) infected several hives with foul brood and when it had fully developed he took a comb of the rotten brood to the Physiological Institute at Breslau, and had it submitted to a microscopical examination by Drs. Cohn and Eidam (22). This examination showed that in every dead larva, and in each foul broody cell, whether the contents were yet white and fluid or brown, tenacious and ropy, there were to be found long oval bodies, which Preuss called "micro-cocci." "Close to and among them, Cohn was the first "to find, with the most powerful of the five microscopes that were used, a "countless number of slender pale rods, joined together, and which he at once "identified as bacteria of the genus *bacillus*. The length of a single rod was "about 6 micromillimetres, but many of them were two and three jointed, so "that these foul brood bacteria microscopically resembled the anthrax bacteria, "though of course they were different physiologically and in the manner in "which they acted as ferments"

It is not surprising when we remember the state of bacteriological knowledge in 1870, that Preuss should have mistaken micrococci for the spores of a bacillus. In 1885, Cheshire & W. Cheyne (23) confirmed Cohn's conclusions and demonstrated that the disease, foul brood, was caused by a bacillus to which they gave the name of *Bacillus alvei*; and they worked out the following requirements of the causal relation of this bacillus to the disease, usually spoken of as Koch's rules:

1. Constant association of this germ (*B. alvei*) with the disease in the larvae of bees.
2. Isolation of the germ from the diseased larvae, and study of the same in pure cultures on various media.
3. Production of the characteristic symptoms of the disease by inoculation of pure cultures.
4. Discovery of the same germ in the re infected larvae. Re-isolation and growth on various media, comparable to that previously isolated.

The infection brought about by Cheshire was accomplished by spraying a particular part of the comb with a culture of *B. alvei* in milk. This part and no other became affected with foul brood. Adult bees were also infected by feeding them with these cultivated bacilli. The experiments of Cheshire and Cheyne convinced everyone, and since that date *B. alvei* has been generally regarded as the causal agent in the production of this disease.

Dickel (24) wrote in 1888 that several species of bacilli were able to produce foul brood. There was one form of the disease which affected the unsealed brood, and another which affected the sealed brood; and even a third, a mixed form, which seemed to be most malignant.

Klamann (25) supported Dickel's researches, but stated that it was not necessary to count more than two kinds of the disease, as there were certainly several other microbes which contributed to the ruin of the hive. Klamann stated that he found seven and was persuaded that he would be able to isolate an even greater number of bacteria from the diseased larvae. It seemed to him certain that *B. alvei* was the most virulent, and that this germ alone was to be considered the cause of foul brood.

SYMPTOMS OF THE DISEASE.

The disease principally affects the larvae. In a healthy comb the young larvae lie at the bottom of the cells, curled up in the shape of the letter C and in color are of a pure pearly whiteness, plump in appearance, with full skin. If examined when the disease is just developing, the affected larvae are usually found to have changed their position. They are no longer curled up, but lie extended in the cell, or move about unnaturally. The bees themselves may at this time or subsequently show symptoms of the disease by a kind of inertness or inactivity which seizes them. As the disease progresses, the young larvae lose their plump appearance, become flabby and die. Then a process of decomposition begins, which is shown by their yellowish appearance. This yellow color turns brown, and if touched with a pin or needle at this time or later, a portion of the putrid mass may be pulled out in a long, ropy, tenacious string, due "to the chitinous aerating sacs and tracheae which do not undergo decomposition at all; and these remaining cause the peculiarity referred to" (26). This ropy mass gradually dries down to the bottom of the cell, leaving nothing but a brown scale which adheres to the wax.

As a rule, the bees do not remove larvae dead from this disease. Instead they become quite inactive, lose their desire to fly, are often seen fanning at the entrance of the hive, which in many cases emits a bad smell. A phase of the disease, which some authors have described as being a different form, is that in which the larvae die after the cells have been capped over. These cappings become darker in colour than those of the healthy brood; then become indented or sunken, and lastly become perforated with irregular holes. By putting a needle into any of these cells the same ropy mass, before described, may be drawn out. If an examination is made from the juice of the larvae at different stages of the disease, the bacillus may be detected; but spores do not form till after death has occurred. The ropy mass contains large numbers of spores, as does also the dry scale.

According to Cheshire (26), the bees themselves become diseased. In a number of cases he obtained the bacillus from the blood of bees from infected hives. Hilbert's examination in 1875 led him to declare that the mature bees in infected hives were liable to be affected. Some writers contradict the statement that the bees themselves are affected by the disease; but they lose sight of the fact that the bees do not die in the hive, but leave it sometime before death occurs.

The queen may become infected. Cheshire (26) demonstrated the presence of bacilli in the ovary of a queen; but he did not make cultures therefrom. W. G. Smith (27) reported that a queen sent to Cheshire and examined by him contained *B. alvei* in both of her ovaries. McKenzie (28) examined five queens from infected hives and succeeded in obtaining bacilli from the ovaries of three. He thinks that their presence there is accidental, as in the case of a queen from a badly diseased hive he was unable to find the bacillus, whilst in a six weeks old queen from a hive in which there were only a few diseased cells, he succeeded in finding it. A queen sent by T. A. Govan (29) to Cowan, the editor of the British Bee Journal, was examined, and *B. alvei* was found in the ovaries. F. F. Ward (30) removed a queen from a diseased hive and placed her in a strong, healthy stock, "which speedily became a mass of corruption." This operation was subsequently repeated with a like result.

I have also myself examined seven queens from diseased hives, and in three cases have had no difficulty in finding the bacillus, and have obtained typical cultures therefrom. The method of examination employed has been the same as that used by McKenzie. The upper surface of the bee is sterilized and cut longtitudinally, and all the internal organs except the ovaries are removed. The surface of the ovaries is then sterilized and a hot needle plunged into the centre and allowed to stay there until it is cold, when it is withdrawn and used to inoculate agar cultures. According to Cheshire (26) the bacilli are found in the eggs. In one examination he says he counted nine bacilli from a half-developed egg taken from the ovary of a queen. McKenzie (28) thinks that this statement requires confirmation, as he was not able to find any infected eggs.

I have myself examined a very large number of eggs at various times. In these examinations three different methods were employed: 1.—The eggs were taken from the cells in which they were laid with sterilized forceps and washed in corrosive sublimate, 1 : 500 crushed and placed on agar plates. In many cases typical growths of *B alvei* developed from eggs thus treated; but as it might be maintained that the eggs were laid in cells previously infected with *B. alvei*, and that the momentary immersion in corrosive sublimate failed to kill all the spores that were upon the exterior, the next lot of eggs 2.—Were crushed between cover-glasses, a small portion transferred into agar, and the remainder on the cover-glass stained by Gram's method. In several instances the bacillus was found in the crushed egg, and in every case the cultural test confirmed the microscopical examination. Again, as this method also might be criticized for the reasons above stated—(3) eggs were imbedded in parraffin and serial sections made and stained by Gram's method No cultural tests were made; but in a few eggs of several hundreds sectioned a bacillus corresponding in its morphological characteristics to *B. alvei* was found. All the eggs examined were taken from hives more or less affected with the disease.

In view of these facts, I am of the opinion that the eggs of bees from diseased hives may in some instances be infected.

CHILLED BROOD.

Chilled brood is sometimes mistaken for foul brood; but the appearance of the former is very different from that of the latter. In the case of chilled brood the larvae turn grey; afterwards the colour darkens, and in the final stages of decomposition it becomes black. No ropiness develops.

A number of writers in various bee periodicals have mistaken chilled brood for foul brood, or they have stated that chilled brood turns to foul brood; but Schirach, as long ago as 1769, clearly distinguished between the two, and McKenzie (28) also performed several experiments in refutation of the statement that if chilled brood is allowed to putrify foul brood may arise *de novo*. He endeavoured to isolate *B. alvei* from chilled brood, but without success. Again, he killed perfectly healthy brood by chilling, and infected some of the cells from a pure culture of *B. alvei*. The chilled brood were allowed to putrify in a moist chamber for several months and examined with the same results, viz : that in the cells in which *B. alvei* was placed it was to be found, but not in any others I have also performed similar experiments and they fully confirm McKenzie's contention. So far *B. alvei* has not been isolated from chilled brood in any stage of decomposition. Canestrini (31) described a case which was in all probability chilled brood and not an infectious malady; but his inoculation experiments failed to establish the pathogenicity of the bacillus, which morphologically resembled *B. megatherium*.

GEOGRAPHICAL DISTRIBUTION.

It has been thought that the disease varies in different countries, that foul brood as it occurs in England is different from foul brood in America; but as no bacteriological evidence has been produced in support of the contention, it is not necessary to argue the question. I have examined diseased larvæ from Canada, from Europe (France, Switzerland, Austria, Germany, Italy and England), Cuba, and 13 States of the Union, ranging from New York to California and from Michigan to Florida, and have succeeded in isolating *B. alvei* from all of them. It is true that some of the cultures show certain differences, but they have not been sufficiently pronounced to constitute even a well marked variety of the species. The pathogenicity of the bacillus no doubt varies in different countries; of that we have abundant evidence, and the possible explanation is given by Bertrand, who thinks that where bees have been kept for many years the disease has existed for a long time and remains in an endemic state; but there has been produced in these countries a race of bees which have acquired a relative immunity, which considerably diminishes the effects of the disease, and enables apiculturists to treat it more easily. In new countries into which the disease has been introduced it rages with great virulence, and remedies giving good results in the older countries are worthless in the new. As an example of this statement, we have the different methods of treatment used in Canada and in Europe.

Bertrand (32) reports the disease as being present in every country in Europe. Benton (33) says that he has never met with the disease during the six years he has kept bees in the Orient. Della Rocca (7) described a terrible epidemic in the Levant in 1780. Bovill (34) says that he has never seen it in Cyprus. In Africa, Feuillebois (35) reports it in Algeria, and Bochatey (81) in Tunis. In Australia it is present in all the colonies, and especially so in New South Wales (36) and South Australia (37). Brickwell (38) reports that New Zealand is full of the disease.

THE ORGANISM.

Bacillus Alvei, Cheshire and W. Cheyne, 1885, from the larvæ of bees suffering from the disease known as foul brood, la loque (Fr.) and faul brut (Ger.).

Morphological Characteristics.—In form the organism is a slender bacillus, with ends slightly pointed and rounded. "In the larval juices it is about $\frac{1}{7,000}$ of an inch in length and $\frac{1}{20,500}$ in breadth. On agar the bacilli vary considerably in size, averaging $\frac{1}{7,260}$ inch, some as small as $\frac{1}{10,000}$ inch, and others as large as $\frac{1}{5,000}$ inch. When they have attained the latter size, division of the rod seems to begin. They are always somewhat pointed at their ends. Their average breadth is $\frac{1}{30,000}$ inch, ranging from $\frac{1}{35,000}$ to $\frac{1}{25,000}$ inch (23). Klamann (25) states that a clear space often appears in bacilli with pointed ends. From agar cultures 24 hours old, at 37° C., the bacilli average 4 μ in length and 1.0 μ in breadth. On gelatine cultures, grown at 22° C., they are somewhat shorter. They grow singly, but occasionally form chains of various length.

Stains.—With the ordinary aniline stains the bacilli colour rather badly—Eisenberg (39) and Klamann (25). The best stains are methelene blue and methyl violet. The bacilli accept Gram's stain, but the spores are not colored by it. I find the most satisfactory stain is methyl violet.

Capsule.—No capsule has been demonstrated by Welch's method.

Flagella.—The bacilli are actively motile and possess a single flagellum at one pole. The motility of the bacillus is quite pronounced in fresh cultures obtained from bouillon, agar and gelatine. The flagella stain by Pitfield's, Loefflers's and Van Ermegen's method.

Spore Formation.—Spores are formed by the bacillus, and are large oval bodies averaging in length $\frac{1}{12,000}$ inch, and in breadth $\frac{1}{23,700}$ of an inch. On agar the spores are arranged in long rows, side by side, and are greater in diameter than the cells from which they are derived. The earliest appearance of spore formation takes place in 41 hours, at 36° C. (Cheyne), but in some cases it is even sooner. The spores are formed in the centre of the rod, and the formation occurs as follows: The rod begins to swell and become spindle-shaped. Occasionally the swelling is more marked at one end than in the centre. The spindle-shape increases in size, and the centre of the swelling gradually ceases to take the stain. The capsule of the spore is apparently formed within the rod and is not merely the outer part of the rod. In three or four hours the rod is seen to have almost or completely disappeared, although parts of the faint outline of the ordinary bacillus may be noticed.

Germination of Spores.—Under favourable conditions the beginning of the germination of the spores takes place in about three hours. The spore loses its oval shape, becomes elongated, and is soon seen to burst through the spore capsule. It then presents the appearance of a short rod, with a pale envelope embracing one end. The rod gradually leaves the spore capsule, and then goes on multiplying as a full grown bacillus. According to Eisenberg (39), the spores are decolorized by the tubercle bacilli stain, but preparations may be obtained by using the Ziehl-Neilsen stain and alcohol for decolorization. The spores also stain by the method of Neisser.

Polymorphism.—Variations in size and shape may be brought about by growth in acid media, or in media containing different sugars. These variations occur also in the same culture, subjected to exactly similar conditions of growth.

Involution Forms.—Abnormal forms are especially abundant when the bacillus is grown on blood serum; peculiar Y-like forms and clubbed shapes are of common occurrence, and relatively few spores are found.

Biological Characters.

Bouillon.—"In meat infusion at the temperature of the body, they grow rapidly, causing muddiness and, after a few days, a slight but not tenacious scum" (23). In bouillon, with a reaction of + .08 (57), at 37° C., there is a slight turbidity in 14 hours, especially noticeable when the tube is shaken. In 24 hours, the liquid is uniformly turbid, with a very fine sediment. In 48 hours, the turbidity increases and a pellicle commences to form. Reaction of the culture at this time, +.07. After 96 hours the broth is clear, with a pellicle, white, rather massive, and somewhat tenacious. There is also much sediment. Reaction, after 10 days' growth,—neutral.

Glycerine Bouillon.—Media with original reaction of +.08. At 37° C., the bouillon becomes slightly turbid in 12 hours, and quite turbid in 24, with a fine, whitish pellicle on surface, which does not extend to the sides of the tube. If the culture is shaken, the pellicle deposits in flaky masses. The reaction is + 1.2. In 36 hours, the turbidity clears, leaving the media bright, with a smooth, thin, tenacious, and white pellicle on the surface. In many cases the pellicle becomes very wrinkled and greasy-looking. At the end of 8 days, the reaction is + 2.2, and the bouillon is several shades darker in colour, but quite clear. The reaction after 14 day's growth is +4.2. At 22° C. the same changes occur but growth is slower. The bacilli are relatively less numerous than in bouillon and are slightly shorter and thicker.

Glucose Bouillon.—With a reaction of + 2.0, at 37° C., the broth is more turbid than plain bouillon after 14 hours' growth; and in 24 hours, the sediment is heavy, and turbidity very marked, but no pellicle. In 48 hours. the media is opaque and cloudy, and the pellicle is beginning to form. In 96 hours the broth is less cloudy, but the sediment is heavier, and a white, thick pellicle is formed. It is often wrinkled, but not quite so much so as that on the glycerine broth. Reaction of broth after 10 days' growth, + 4.6. The bacilli are occasionally clubbed and y-like forms may occur. They average 5 μ in length and may be slightly curved.

Lactose Bouillon.—With a reaction of + 1.06, at 37° C., the growth resembles that of plain bouillon for the first 24 hours; but at the end of 48 hours, it is more turbid. In 96 hours, a tenacious pellicle forms, less massive than that on Glucose broth. Reaction after 10 days' growth, + 2.4. The bacilli average 3.5 μ in length.

Saccharose Bouillon.—With a reaction of + 1.0, at 37° C., the turbidity and sediment are heavier than any of the other bouillons. In 48 hours the broth is quite opaque and whitish looking. A heavy sediment is then present and pellicle formation is just beginning. In 96 hours, the cloudiness is about the same, but there is an increase of sediment and the pellicle is thin and membranous. Reaction of media after 10 days' growth, + 4.04. The bacilli average 5 μ in length.

Gelatine Plates.—At 22° C. in 24-36 hours, the colonies are small, round, oval, or lozenge-shaped, with peculiar projections or shoots from one end of the colony, giving it a pear-shaped, or tadpole-like appearance, according to the amount of development of the projection. In many cases, several of these outgrowths occur from different portions of the colony. By placing a cover glass on the surface of the gelatine and using objective 7, the bacilli may be seen moving around and around the colony and to and fro along the projections. At the end of 48 hours, the colonies are larger. Fine processes or projections are shooting out into the gelatine in all directions, forming peculiar figures in circles or club-line forms. "It is impossible," says Cheyne, "to give a proper idea of the appearance of the growth. The forms assumed are the most beautifully shaped I have ever seen; but they are very numerous, always retaining the tendency to form curves and circles." After a time the gelatine is liquefied and the beautiful appearance of the colony is destroyed by the liquefaction of the gelatine.

These peculiar shaped colonies are most typical when the germ is taken from the diseased larvæ. After prolonged cultivation on various kinds of media, there is a tendency for the colonies to become round, and the peculiar branching forms are not seen in such numbers. The composition of the gelatine also seems to make a difference in the appearance of the colonies. In gelatine containing 12 per cent. gelatine the processes are not so long. The same effect may be brought about by using more peptone in the composition of the media.

Gelatine Tubes.—In stick cultures at 20° C. growth occurs all along the line of puncture. On the surface, delicate branching or ramifying growth occurs in three days. These outgrowths soon run together and the gelatine is liquefied, first around the line of puncture, and in 5 days extends over the whole surface. The growth in the depth of the gelatine occurs as a whitish streak all along the needle track; and from this, numerous shoots and growths branch out into the gelatine in all directions, giving a haziness to the appearance of the gelatine, which then begins to liquefy. If the inoculation is a heavy one, the shoots are coarse and may have club-shaped extremities, and from these swollen ends fresh shoots may start. Cheyne obtained the most characteristic growth in gelatine containing 3 per cent. of peptone, as well as 10 per cent. gelatine. The whole tube is liquefied in from 2·4 weeks' growth. The liquid becomes yellowish in color and gives off a peculiar odor. Klamaunn states that in gelatine acidified with lactic acid the growth is slow and long threads are formed.

Gelatine Streak Cultures.—In gelatine streak cultures the appearance is very similar to what one sees in stick cultures. The bacilli first grow along the line of inoculation; and then throw out shoots into the surrounding gelatine, producing the appearance noted in the stick culture. The bacilli move to and fro along the channels of liquefied gelatine.

Agar plates.—On agar plates at 37° C., the colonies at the end of eight hours are small and burr-like, with spines protruding in all directions, giving the colony the appearance of a sea-urchin. In some cases the projections are from one side or end. At the end of 12 hours, the colonies have well-defined projections, visible to the naked eye. The colonies in the depths of the agar are more spiny, the processes being much shorter. On agar plates streaked with a light inoculation, most beautiful forms occur. The growth of the bacilli spreads over the surface and branches repeatedly, giving the appearance of seaweed. This appearance is distinctively characteristic; and as the growth is very rapid, this method commends itself for making a quick diagnosis of the presence of the bacillus in larvæ supposed to be diseased.

Potato cultures.—On potatoes the growth differs considerably, according to the reaction and age of the potato. Sometimes a brownish wrinkled growth forms, which gives off a peculiar odor; at other times a dryish yellow layer appears. "The bacilli grow very slowly indeed at 20° C." (Cheyne 23.) Even at 37° C. they grow slowly.

Milk.—In milk at 37° C., coagulation of the casein occurs in three days. The milk becomes yellowish and gives off a characteristic odor. After several week's growth, the curd is digested and a whey-like fluid remains.

Blood serum.—On blood serum at 37° C, the growth is rather slow and polymorphic forms are common. "Very long filaments are formed" (23). These long forms may be from 5 to 10 times as long as the average bacillus growing on gelatine, and consist of single cells. The filaments are often wavy or twisted and of unequal thickness. The extremities of the long, bent rods are often clubbed; and y-forms are numerous. Spores are formed very sparingly, and the blood serum is liquefied.

Synthetic media (Uschinsky).—In Uschinsky's medium no growth occurs; but if the medium is neutralized, good growth ensues. The bacilli occur in threads and a pellicle is formed.

Dunham's Solution. The bacilli are small when grown in this solution; No threads form; but there is a slight indol reaction after nine days' growth.

Relation to Free Oxygen. Cheyne states that the germs grow most rapidly on the surface of agar and arrange themselves side by side; and they produce spores in this position after a few days' growth. Eisenberg (39) says nothing under the head of aerobiosis. Howard (40) writes that, "It grows best under anaerobic conditions; is a facultative aerobe; grows under the mica plate; and in the presence of oxygen the growth is slight and slow." Howard also states that under anaerobic conditions it emits a foul odour resembling that of foul brood. It will be thus seen that Cheyne and Howard do not agree on this point. The former author also says that the characteristic odour is given off under aerobic conditions, whilst Howard states that this smell is emitted under anaerobic conditions. Further, Cheyne states that the bacilli grow with great rapidity on the surface of agar, whereas

Howard obtains his best growth under the mica plate, which does not give complete anaerobiosis. Howard's conclusions are thus at variance with Cheyne's, and my own results fully corroborate those of the latter author.

Howard states that the vitality of the spores of *B. alvei* is destroyed when exposed to atmospheric air from 24 to 36 hours. In making his experiments he took sterilized road dust and mixed it with the dry foul brood masses from several cells which were previously dissolved is distilled water. The mixture was worked dry, and spread on sheets of paper, and trial cultures were made immediately and at intervals of every twelve hours for three days; and according to his results no growth occurred after 36 hours In giving these results, Howard does not state whether he exposed the spores to sunlight or diffused light; nor does he mention the age of the dry foul brood masses, which he used from several cells. These are points of considerable importance, for as everyone knows the disinfecting power of direct sunlight is much greater than diffused light, and the vitality of the spores from foul brood masses of different ages varies considerably. This, I may add, has been clearly shown by some of my experiments, subsequently described. In my experiments, the spores obtained from a pure culture on the surface of agar, were spread on cover glasses and placed in a glass chamber, so arranged that a current of air was constantly circulating over them. This chamber was exposed to the ordinary light of a room with six large windows, and a cover glass was taken out every 24 hours and tested, to see if the spores would grow. This experiment was continued for one month and at the end of that time the spores still germinated rapidly. In another experiment, spores spread on cover glasses were exposed to a very diffused light, simulating as far as possible the amount of light which would enter a hive. Cover glasses were taken out from time to time and transferred to agar, in order to ascertain if the spores were alive or not. The experiment was begun two years and four months ago and from the last cover glass taken and placed upon the surface of an agar plate a copious and typical growth of *B. alvei* was obtained. Further, thin strips of filter paper, plunged into a bouillon culture and allowed to dry, were threaded on a wire suspended in a wire basket and so exposed that the air could freely circulate around them in the ordinary light of a room. Trial cultures were made at intervals, and at the expiration of 6 months the spores from the paper germinated when the strips were placed on the surface of agar.

Again, a drop of bouillon containing spores was placed in a sterile tube and allowed to dry; and at the expiration of 124 hours (36 of which were in sunlight at a temperature varying from 30° 37° C) sterile bouillon was added. The tubes were then placed in the incubator, and in less than 24 hours a good growth of the germs had taken place.

From these experiments it will be seen that the results are directly at variance with Howard's statement, as they go to show that the vitality of the spores of *B. alvei* is not destroyed by exposure to atmospheric air, with or without sunlight, for even a much longer time than 24-36 hours.

With regard to the aerobiosis of this bacillus, good growth has been obtained in an atmosphere of hydrogen by Novy's method. Buchner's method also gave good results. The growths in the various media are very similar to those produced under aerobic conditions, but with this difference, that the surface growths are, as a rule, whiter in the hydrogen atmosphere. In illuminating gas (water gas) no growth occured; but the spores were not destroyed by the action of the gas; for when the gas was let out of the Novy jar, good growth ensued on all cultures. In acetylene gas, a restricted growth occurred. In fermentation tubes, growth occurred both in the open and in the closed arm of the tubes. No gas was formed, the bouillon in the closed arm was uniformly turbid. Thus *B. alvei* is a facultative anaerobe.

Production of Alkali In ordinary bouillon a slight amount of ammonia is formed. Control bouillon did not give the Nessler test. In glycerine and the sugar bouillons, there is no trace of ammonia. Cheyne's cultures are faintly alkaline, both before and after inoculation in meat infusion. Klamann states that ammonia is produced.

Acids formed A varying amount of acid is formed. All the sugar bouillons give an acid reacton.

Formation of Pigment. On potatoes, a yellowish growth is produced; on all other media, the surface growth is white.

Development of odours Cheyne states that gelatine cultures give off an odour of stale, but not ammoniacal urine, or what may be better described as a shrimpy smell; and this peculiar odour has been formed by Cheshire to be distinctive of diseased larvae. Klamann and Howard both state that a peculiar odour resembling that of the diseased larvae may be noticed in artificial cultures.

The Effects of Desiccation. I have already noticed, under the head of "Relation to Free Oxygen," that the spores of *B. alvei* have considerable vitality in withstanding desiccation. My experiments prove conclusively that the spores are extremely hard to kill by desiccation and in this respect resemble those of anthrax, which are known to resist thorough desiccation for a number of years. One experiment which shewed this characteristic was as follows: An agar plate completely covered with a typical growth of *B. alvei* was allowed to dry out completely, and was left exposed to the ordinary light of the room for 7 months, and at the end of that time, a portion of the film was scraped off with a knife, placed on suitable medium and incubated, with the result that a typical growth immediately ensued.

Spores on cover glasses were exposed to September sunlight (Latitude 43) for varying periods of time, and growth occurred after 4, 6 and 7 hour's exposure. The age of the spores varied from 5 days to 18 months; and spores 3 months old were not killed by 7 hours' exposure.

THERMAL RELATIONS.

Maximum for Growth. The maximum for growth is about 47°C. At 44°C., good growth occurs; but at 50°C., growth ceases. Experiments on maximum for growth were performed on germs isolated from a number of different places, and little or no difference was noticed in their behaviour when incubated at the temperatures mentioned.

Optimum for Growth. The optimum for growth is about 37.5°C. for all media except gelatine. This has been determined by Cheyne & Eisenberg (39). On gelatine the best results are, of course, obtained from higher temperatures; but as 10% gelatine melts at about 24°C., 22°C cannot be exceeded.

Minimum for Growth. Cheyne says that the bacilli do not grow below 16°C. I have, however, occasionally obtained growth at 14°C. on the surface of agar; but it has been extremely slow. The spores will not germinate at this temperature. No difference, under this head, is apparent in germs obtained from different countries.

Thermal Death Point. This is a very important matter, because in the heating of wax and honey from colonies suffering with foul brood, it is necessary to know the temperature that will destroy spores and thus prevent the infection of other bees; and unfortunately a considerable discrepancy exists in the results of experiments to determine the thermal death point of the bacillus, accounted for in part by the different methods used by different investigators.

McKenzie (28) found the thermal death point by suspending silk threads saturated in a beef broth culture of *B. alvei* containing spores. The threads were allowed to dry, and introduced into melted wax, and left therein for a definite time, at a fixed temperature. At the end of that time, the thread was introduced into melted agar and thoroughly shaken so as to separate the wax from the threads. The cultures thus made were rapidly cooled, and the tubes placed in the incubator at 37°C. The following are his results :

At 100°C. for ¼ of an hour,	growth.	At 90°C. for ½ hour,	growth.
" " ½ " "	growth.	" " 1 "	growth.
" " 1 hour,	growth.	" " 2 hours,	growth.
" " 1½ hours,	growth.	" " 3 "	no growth.
" " 2 "	growth.	" " 4 "	no growth.
" " 3½ "	no growth.		

A temperature of 50°C. did not destroy the spores in 24 hours. These experiments were repeated with the same results, which results were criticised by Corneil (28), who claimed that the heat to which the bacteria were exposed in melted wax was not moist but dry heat, and consequently that the wax had to be heated to a high temperature and for a long time in order to destroy the spores. According to the testimony of two prominent foundation makers, the wax during the refining and purifying process reaches a temperature of quite or nearly 100°C. for a short time. During the sheeting, however, it does not reach a temperature much above the melting point, say 79°C. Two other foundation makers, Dadant & Hunt (41), state that, in refining, the wax is heated for some time to 100°C., and is kept liquid for 24 hours ; so McKenzie thinks that if these temperatures are reached in the making, there is little danger of foul brood from comb foundation, as the specific gravity of bacteria in the melted wax is so great that throughout the process of manufacture the bacteria tend to fall to the bottom. Sternberg (42) states that the spores require for their destruction a temperature of 100°C. for four minutes (determined in 1887); but there is no statement as to the age of the spores. In Howard's experiments (40) tubes of liquid gelatine containing spores of *B. alvei* were placed in an open vessel of boiling water and allowed to remain therein for a definite time—" in all probability the water did not reach boiling point "—and trial cultures were made at stated intervals, with the following results :

After 15 minutes—	growth.
" 30 "	"
" 45 "	"
" 50 "	no growth.
" 60 "	"

His trial cultures were on potato and gelatine ; but no statement is made regarding the age of the spores, where they were from, or the temperature at which they were incubated. It is, however, evident that they were not given the most favourable conditions for growth.

I have myself performed the following experiments on the thermal death point of the spores :

Method. Test tubes containing bouillon were placed in boiling water. Three loopfuls of culture were introduced into each of the tubes ; and tubes, withdrawn from the boiling water at stated intervals, were cooled and incubated.

Results 1. Spores from a seven months old culture in bouillon were killed at a temperature of 100° in 1 hour and 20 minutes.

2. Spores from a 2½ months old culture on agar were killed in two hours and a half.

3. Spores from agar nine days old,—slight growth after 2 hours and 45 minutes ; no growth after three hours.

4. Spores 14 days old and 21 days old,—in each case after two hours boiling, one of the duplicate tubes formed a growth ; another after 2½ hours, whilst the remainder had no growth. All were killed in 3 hours.

I used also fine capillary glass tubes. A suspension of the spores in water was drawn up into sterile tubes, which were then sealed at both ends, The tubes were placed in boiling water and withdrawn at stated intervals. The contents of the tubes were then introduced into agar, which was incubated at 37°C. ; and great care was taken to have a suspension of the spores by filtering them through glass wool.

The results were : With a temperature of 98°C. (about the boiling point in this locality), spores from a 7 days' old culture on agar were killed in 2¼ hours ; and spores from agar 9 days old were killed in 3 hours.

Another experiment was made to determine the thermal death point in honey. The honey was of two kinds, clover and buckwheat. The former had a specific gravity of 1.042 at 60°C. and contained 0.057% of formic acid, while the latter had a specific gravity of 1.040 at 60°C. and contained 0.170% of formic acid. The spores used were from agar three weeks old, and three methods were followed :

1. Silk threads with dry spores thereon ; 2. Test tubes containing honey with a heavy inoculation of spores ; 3. Capillary tubes containing a suspension of spores in distilled water. The spores used were not filtered through sterile glass wool, as it seemed desirable to imitate as far as possible the conditions met with in infected honey. The following are the results :

1. *Silk threads with dried spores, from an agar culture two weeks old.*

Time.	Temperature.	Result.
15 minutes	115°G.	growth.
30 "	113 "	"
45 "	115 "	"
60 "	113 "	"
1 hour 15 minutes	114 "	"
1 " 30 "	115 "	"
1 " 45 "	115 "	"
2 hours	114 "	"
2 " 15 minutes	116 "	"
2 " 30 "	115 "	"
2 " 46 "	115 "	no growth.

2. *Tubes containing honey and spores mixed together.*

30 minutes	115°C.	growth.
45 "	114 "	"
60 "	114 "	"
1 hour 15 minutes	114 "	"
1 " 30 "	114 "	"
1 " 45 "	115 "	"

2 hours	115°C	growth.
2 " 15 minutes	116 "	"
2 " 30 "	115 "	no growth.
2 " 45 "	115 "	"

3. *Capillary tubes with spores in distilled water.*

30 minutes	114°C.	growth.
1 hour	114 "	"
1 " 30 minutes	114 "	"
2 hours	114 "	"
2 " 15 minutes	115 "	"
2 " 30 "	115 "	"
2 " 45 "	115 "	no growth.

The temperatures were taken in a large vessel containing 10 pounds of boiling honey. The experiment was repeated, using buckwheat honey instead of clover and with like results.

Relation to Light. A few experiments were made to ascertain the behaviour of spores toward light. Coverglasses spread with spores and dried, were exposed to bright sunlight during the month of February. The exposure was in the open air and the glasses were on black tile. The temperature varied from—12° C. to—22° C After exposure, the glasses were placed film side downwards on agar plates, and then incubated at 37° C.

	Time.	Result.
Results—	3 hours sunlight.	Abundant growth in 16 hours
	6 " "	" " " "
	9 " "	" " " "

These experiments were repeated in September, when the outside temperature varied from 24° to 30° C., with the result, that there was growth after 4, 6, and 7 hours' exposure.

Agar plates exposed after inoculation showed great differences For instances, spores 21 days old was killed by 5 hours' exposure, whilst plates made the day after with spores 2 months and 21 days old, required 7 hours' exposure. Spores 10 days old showed no growth after 5 hours' exposure; and spores 5 days old, no growth after 6 hours' exposure. From a large number of determinations, the average length of exposure necessary to kill spores within the above range of temperature was found to be 5 hours.

Vitality on various media. The cultures seem to live longer on agar than in liquid media. The vitality of old gelatine and bouillon cultures seems to be lessened by the products of the bacilli growing in these media. The spores taken from these sources have also decreased resisting power.

Effect of growth on reaction of media. Ordinary bouillon becomes slightly more alkaline as growth proceeds, the presence of ammonia being detected by Nessler's reagent; but control bouillon does not give the reaction. In bouillon, with the addition of glycerine and various sugars, the acidity of the media is increased, but more in the case of glucose broth than in any other. In these experiments accurate titration was made with phenolphtalein as indicator. Cheyne tried the reaction, "making the infusions faintly alkaline, and after the growth of this organism in it, it is faintly alkaline."

Sensitiveness to Antiseptics and Germicides. This subject is taken up in connection with the chemical remedies used for the disease.

Pathogenesis. Besides being pathogenic to the larvae of bees, Cheyne has inoculated two mice and one rabbit with spore-bearing cultivations without effect. "Half a syringeful of a spore-bearing cultivation injected into the dorsal subcutaneous tissue of each of two mice resulted in the death of one of them in 23 hours, while the other seemed unaffected. In the case of the mouse which died, the seat of injection and the neighbouring cellular tissue was found to be very œdematous; but no microscopic changes were apparent in the internal organs. Numerous bacilli were found in the the œdematous liquid, as also a number of spores which had sprouted; and there were likewise a few bacilli in the blood taken from the heart. This was proved by cultivation as well as by microscopic examination. On examining sections of various organs no morbid changes were found, and only a few bacilli were seen in the blood vessels. A syringeful of the same culture was injected into a guinea pig; and the animal died 6 days later, with extensive necrosis of the muscular tissue and skin; and cheesy looking patches were distributed through it, but there was no true pus. On making sections of the necrosed tissue, numerous bacilli, apparently *B. alvei*, were seen; but there were also other bacilli and micrococci. No micro-organisms were seen in the internal organs. It thus remains questionable whether the necrosis was due to *B. alvei* or not, more especially as I have since injected three guinea pigs subcutaneously with spore bearing cultivation, but without effect.

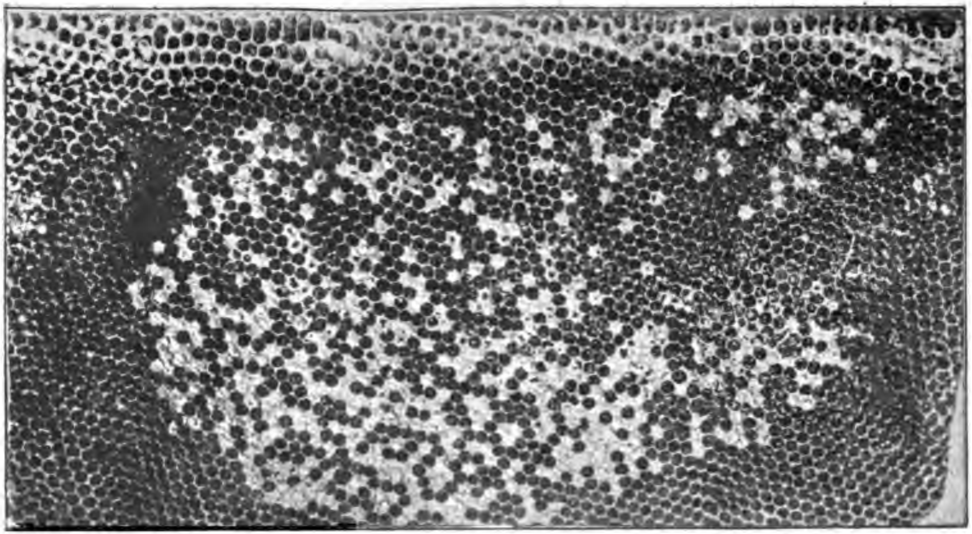
"The effect of feeding flies with material containing spores results in death of the flies, and bacilli were found in its juices as shewn by the microscopic examination and cultivation. Cockroaches were not killed" (28).

Fly blow larvae fed for three days on spores were not killed. With regard to the prevalence of the disease amongst wild bees, very little can be found on this subject in bee literature, but a correspondent of the *British Bee Journal* (43) found the disease among wild bee larvae in a tree, recognising it by the smell from the entrance and also from the appearance of the brood in the combs. The correspondent remarks that this tree had probably in former years been the cause of a great deal of trouble to neighbouring bee keepers. In all probability the disease is present among the various varieties of wild bees and wasps. Knight (54) mentions an epedemic among wasps in 1807; Kirby & Spence (55) another in 1815; and Bevan (13) one in 1824; but in none of these cases was any positive evidence given to show the epidemic was foul brood.

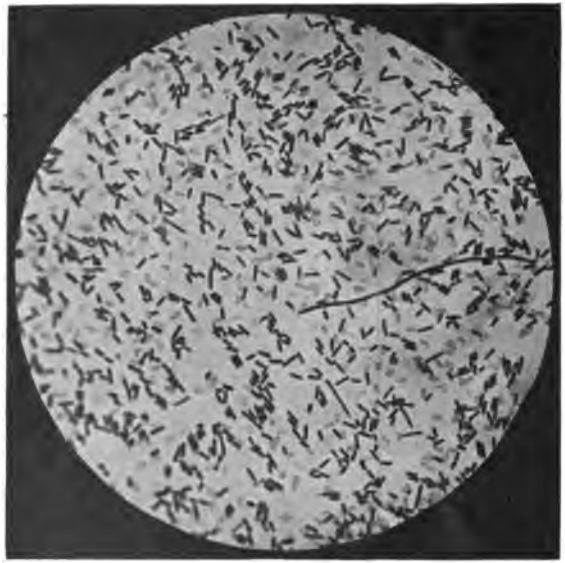
ECONOMIC ASPECTS.

Losses. Della Rocca (loc. cit.) in 1790 stated that the whole of the bees in the Island of Syra were carried away during 1777 to 1780 by the disease. Dzierzon (46) relates his losses from the disease. In 1868 he lost his entire apairy of 500 colonies from it. In Switzerland, the disease, at times, is extremely bad. Bertrand's apiaries have suffered severely, and the German papers make constant reference to its devastation. In England, Cowan (4) thinks that the "only visible hindrance to the rapid expansion of the bee industry is the prevalence of this pestilential disease which is so rapidly spreading over the country as to make bee-keeping a hazardous occupation"; and again, (47) "So rapidly has foul brood spread by contagion that in one season, unless precautions are taken, a whole neighborhood may become seriously infected, and the chances of successful beekeeping seriously imperilled, if not utterly destroyed.

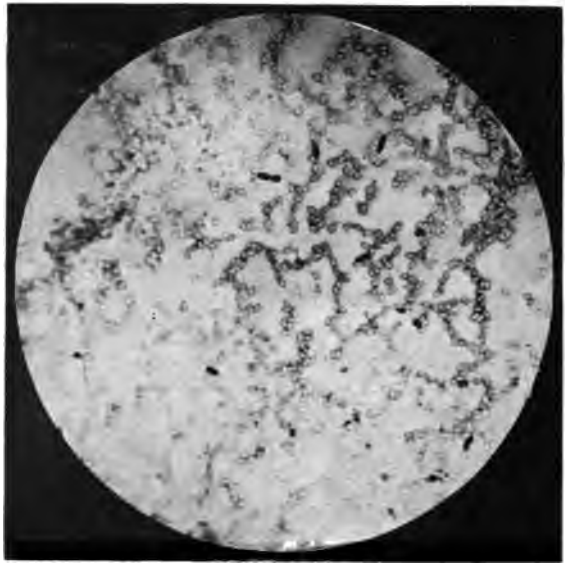
The committee on the Beekeeping Industry and Foul Brood in the United Kingdom, report that the destruction of stock by foul brood and the



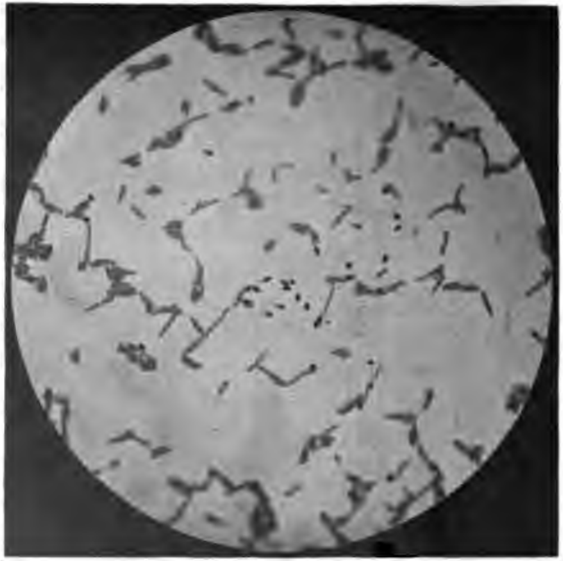
A diseased comb, (after N. E. France), showing sunken and darkened cappings. Also many cells with holes in the cappings.



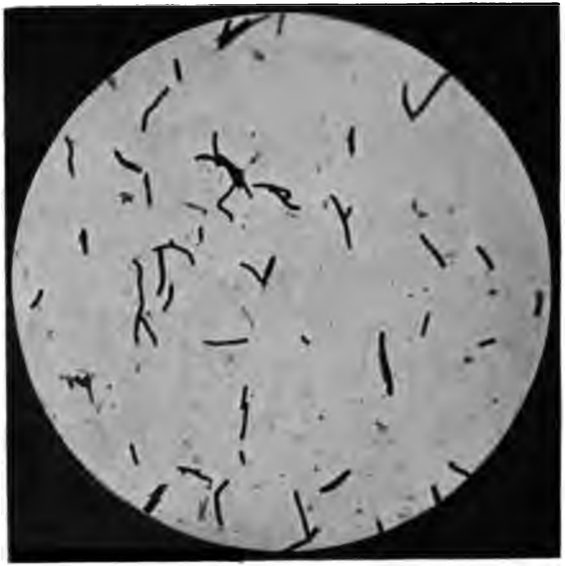
B. alvei and spores x 1000, from gelatine 7 days old at 20° C., stained with methyl violet. (Original.)



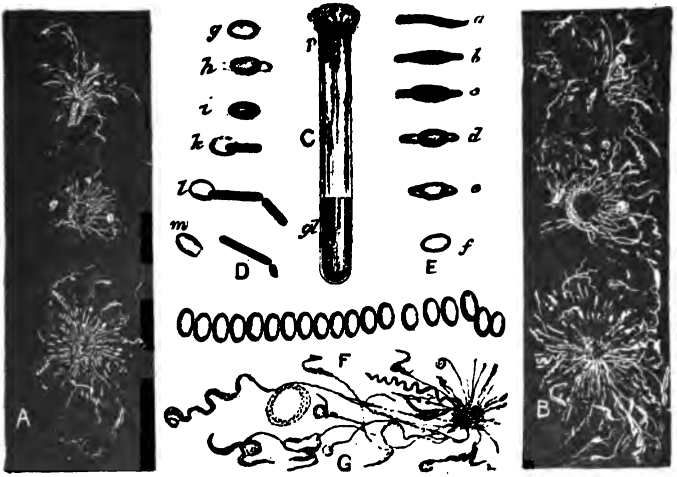
Spores of *B. alvei* x 1000, from agar 3 weeks old at 37° C., stained with methyl violet. (Original.)



B. alvei and spores x 1000, from agar 10 days at 37° C., stained by Moeller's method. (Original.)



B. alvei x 1000, from blood serum 7 days old at 37° C., stained with methyl violet. (Original.)



Cultures of *B. alvei* (after Cheyne). A. Colonies on the surface of gelatine (6 diameters). B. The same colonies 24 hours later. C. Culture tube; gl. gelatine; p. cotton wool plug. D. Spore becoming bacillus (1800 diameters.) E. Bacillus becoming a spore. F. Spores in line, taken from a gelatine culture. G. Colony developing.

discouragement arising therefrom is one of the two influences that retard the development of the bee industry.

In the United States, serious harm has been done, but no definite statistics can be cited. The disease causes great losses and several States have enacted laws for the prevention of the disease, making it a legal offence for a person to keep in his apiary a colony of bees affected with foul brood.

In Canada, the Ontario Foul Brood Inspector (56) reports in the years 1890 1892 inclusive, 622 apiaries inspected and 2,395 cases; in the years 1893-1898, 527 apiaries inspected and the disease present in 212, or about 40 per cent.

In New Zealand and Australia, the disease is looked upon as being very wide spread. It will thus be seen that wherever bees are kept, serious losses are caused annually by this disease.

Natural method of Infection. With regard to the natural methods of infection, a good deal depends on the natural predisposition of the bees to disease and the state of health of the colony. Weak, sickly, or badly nourished bees are as a rule the most susceptible. We must also remember that germs themselves vary in their ability to produce disease. As in diphtheria, we may get a light or severe type of the disease; so also in foul brood, we may have a light or a severe attack; but the facts demonstrating the variability of this capacity are not well known; I have, however, noticed that after prolonged cultivation of *B. alvei* in which more than 30 transfers have been made, and the bacteria with spores have been given to bees in syrup, the virulence of the germ has seemed to be considerably impaired. In one case the colony experimented with was rather weak, was confined to the hive all day, and allowed flight only in the evening, and the spores were given in large quantities in syrup every day, nevertheless it was several weeks before the disease established itself, and then only in a light form So we may have mild or severe epidemics and the liability to take the disease may be increased by chilling the bees or otherwise unfavourably modifying their metabolism; and in all such cases, they succumb more easily to the disease than when in a normal, healthy condition.

With regard to the manner in which the disease is carried from hive to hive, Cheshire (26) thinks that the larvae are most usually affected by the antennae of the nurse bees, and also that the tramp of the bees frequently detaches numbers of spores, which fly about in the air and settle here and there, often where they take effect. I think that in comparison with other diseases which are air borne there is usually not very much danger from this cause in the case of *B. alvei* The spores are generally found in very sticky surroundings, which, even if dry, serves to fix and keep them *in situ*. Cheshire also states that he has not found the bacillus from honey or pollen in infected hives. This statement, however, is directly contradicted by the experience of practical bee-keepers and others. I have myself repeatedly found *B. alvei* in capped honey cells, and in the pollen masses found in diseased hives, the examination in the former case having been made by removing the capping with sterilized forceps and plunging a heated platinum needle into it and then putting the needle into melted agar, from which plates were poured, cooled and incubated.

Probably the chief method of carrying the disease from one hive to another is by the bees from healthy hives robbing colonies that have become weak and diseased. In such cases the robbers carry with them the germs of the disease. There is likely nothing to be feared from using wax foundation from the regular makers; for, as we have already stated, the wax, in the process of making, is subjected to a temperature sufficiently high to kill any spores that may be present.

I may add that I found spores of *B. alvei* in two samples of wax sent me by R. F. Holtermann of the *Canadian Bee Journal*, but both samples were from hives which were very badly infected with the disease.

In 1897, about ten pounds of wax was infected with large numbers of spores grown upon agar. The wax was cut up into small pieces, and heated at a low temperature, only just sufficient to melt it; and as McKenzie (28) had shown that the spores settled to the bottom, the wax was vigorously stirred from the time the spores were added until it had set again. The wax, thus infected, was sent to Holtermann for foundation-making. He manufactured it by the usual process of melting and gave the foundation made from it to bees, and no foul brood developed in the colony supplied with it during the years 1897 and 1898. The probability is that the spores are fixed in the wax, and are thus unable to infect the bees.

Healthy bees may pick up spores of *B. alvei* from flowers previously visited by diseased bees; wasps, which are noted robbers, may also carry the disease, and thus infect a locality.

The very large traffic in bees and bee-keeping supplies where agriculture is carried on, probably favors the spread of the disease. In fact, many instances are cited in bee journals of infection carried from one locality to another by the importation of bees and bee supplies.

Persons manipulating diseased hives and then examining healthy ones may be the means of spreading the disease. The practice of using a knife for cutting out diseased comb and then using the same knife for work amongst healthy comb (which I have seen done) is by no means wise, as the spores may thus be transferred from diseased to healthy hives. Cowan (4) observes that beekeepers who have not succeeded with their bees in consequence of foul brood have been known to sell by auction hives in which the bees have died. In such cases the purchasers are usually beginners who have no idea of the danger they are incurring.

Conditions favoring the spread of the Disease, Besides the weak or badly nourished condition in which bees may be, and lack of other hygenic conditions which favour the spread of this disease, great humidity in winter is said to be favourable and probably great heat is also conducive. (45.)

Predisposition of Varieties. No definite statements can be made as to the predisposition of various races to this disease. Quinby (49) says that black bees are more subject to foul brood than Italians. Aspinall (51) also affirms that common bees are more liable to the disease than Italians, but de Layens (47) states that Italians are more easily infected than black bees. (See also page 17.)

REMEDIES.

Three remedies have been tried:

1. Stamping out.
2. Starvation.
3. Treatment by chemicals: (a) by feeding chemicals in food; (b) by putting certain chemical substances into the hive and allowing them to evaporate at the temperature of the hive. This latter method may be regarded as rather preventitive than curative.

1. *Stamping out Method.* By the stamping out method all affected bees, combs and frames are destroyed, and the hives thoroughly disinfected. Cowan (4) thinks that if foul brood were under government inspection, and all cases promptly dealt with by destruction, the disease could be stamped out. The British Bee-Keepers' Association has asked the Board of Agriculture to secure legislation on this line, because it thinks that in this way the trouble would be removed and the industry would receive an impetus which would benefit bee-keepers, farmers and fruit growers.

The earliest advocate of this system was Della Rocca (18), who maintained "in extreme cases that it was necessary to burn everything without pity, as there was no other resource." Since Della Rocca's time, this method has been frequently resorted to in severe cases that would not yield to treatment either by starvation or by the use of chemicals; but to have any lasting effect, it would have to be universally carried out, and would involve the difficult question of compensation.

2. *Starvation Methods.* The starvation method was first proposed by Schirach (3) who advised that the combs be removed and bees allowed to fast during two days, and then be placed upon clean new comb, and fed on a syrup prepared with a little hot water mixed with honey, nutmeg and saffron.

Since Schirach's time different modifications of this method have been made, and it has been largely used in the United States and Canada, whilst in Europe treatment by medicated syrups has been more in vogue. In 1879 L. C. Root (58) gave his approval to this method, but he advised that the bees be confined in a cool, dark place for 24 hours, in order that all the honey which they carried with them might be consumed, and that the bees be then put into a hive filled with healthy comb or foundation and the condemned hive scalded with boiling water and thoroughly scraped. At a later date McEvoy (44), the Ontario Provincial foul brood inspector, introduced another modification and has himself described his method as follows: "In the "honey season, when the bees are gathering freely, remove the combs in the "evening and shake the bees into their own hives; give them frames with "comb foundation starters on and let them build comb for four days. The "bees will make the starters into comb during the four days and store the "diseased honey in them, which they took with them from the old comb. "Then in the evening of the fourth day take out the new combs and give "them comb foundation to work out, and then the cure will be complete. "By this method of treatment all the diseased honey is removed from the "bees before the full sheets of foundation are worked out. All the old foul "brood combs must be burned or made into wax after they are removed "from the hives, and all the new combs made out of the starters during the "four days must be burned or made into wax, on account of the diseased "honey that would be stored in them.

"All the curing or treating of diseased colonies should be done in the "evening, so as not to have any robbing done or cause any of the bees from "the diseased colonies to mix and go with bees of sound colonies. By doing "all the work in the evening it gives the bees a chance to settle down nicely "before morning and then there is no confusion or trouble.

"This same method of curing colonies of foul brood can be carried on at "any time from May to October, when the bees are not gathering any honey "by feeding plenty of maple syrup in the evenings to take the place of the "honey flow.

"It will set the bees robbing and spread the disease to work with foul "broody colonies in warm days, when bees are not gathering honey, and for that "reason all work must be done in the evenings, when no bees are flying.

"Where the diseased colonies are weak in bees, put the bees in two, "three or four together, so as to get a good sized swarm to start the cure with, "as it does not pay to spend time fussing with little weak colonies.

"When the bees are not gathering honey, any apiary can be cured of "foul brood by removing the diseased combs in the evening, and giving the "bees frames with comb foundation starters on. Then, also, in the evening "feed the bees plenty of sugar syrup, and they will draw out the foundation "and store the diseased honey which they took with them from the old combs; "in the fourth evening remove the new combs made out of the starters and "give the bees full sheets of comb foundation and feed plenty of sugar syrup "each evening until every colony is in first-class order.

"Make the syrup out of granulated sugar and put one pound of water to "every two pounds of sugar, and then bring it to a boil As previously "stated, all the old combs must be burned or made into wax when removed "from the hives, and so must all the new combs made during the four days.

"The empty hives that had foul brood in them do not need any disin-"fectant in any way. I have handled many hundreds of colonies in the Pro-"vince of Ontario and cured them of foul brood without getting a single hive "scalded or disinfected in any way, and these colonies are cured right in the "same old hives."

McEvoy positively states that "No colony can be cured of foul brood by "the use of any drug. All the old combs must be removed from every dis-"eased colony and the hive got away from the bees before brood rearing is "commenced in the new clean combs."

Howard (40) is most emphatically opposed to the drug treatment. "I regard," says he, "the use of any and all drugs in the treatment of foul brood as a useless waste of time and material, wholly ineffectual, inviting ruin and total loss of bees. Any method which has not for its object the entire removal of all infectious material beyond the reach of both bees and brood *will* prove detrimental and destructive and surely encourage the recurrence of the disease."

A. I. Root (45) says that "The starvation plan in connection with burning the combs and frames and boiling the hives has worked best in treating foul brood. It never reappeared after such treatment, though it did in all cases where the hives were not boiled, thus confirming the theory or fact of spores."

These two authors, therefore, go further than McEvoy in both advising the disinfection of the hives.

McEvoy (56), however, admits that his method as described above cannot be used for every case. His reports frequently refer to burned colonies; and he acknowledges that his method does not always cure. In 1890 he used the expression, "600 cases of foul brood and over 360 cured"; and again in a subsequent report, after mentioning the number of cases, he added the words, "*mostly cured*"

In a personal communication, M. Bertrand of Nyon, Switzerland, states that he does not believe in and will not recommend in his periodical (*Revue Internationale d' Apiculture*) the starvation method as used in America.

3. *Treatment by Chemicals*—In the treatment of bees by chemicals, we assume that such substances as are used are employed as antiseptics, and that their efficiency is due to the fact that they destroy the bacillus or prevent the germination of the spores, and thus bring about an internal disinfection; but we must remember that many of the substances used are more poisonous in their effects upon the cells of the bee than upon *B. alvei*. As is well known, quinine is frequently used as a specific for malaria; and in such cases the

cure is effected by the intervention of the body cell. The effectiveness of the remedy is due to the fact that it acts as a stimulus and exalts the natural forces of the body.

Whether the drugs used in the treatment of foul brood act antiseptically or by stimulating the cells of the bee and making them more active to ward off the disease, is a matter of doubt; but it must be admitted that certain drugs do seem to effect a cure, and some of them are regarded as specifics by practical beekeepers.

In taking up the different methods of chemical treatment, I shall as far as possible describe them in the chronological order.

(1) *Carbolic acid.* Carbolic acid was first proposed by Butlerow (52), who recommended one part of acid to 600 of syrup, this proportion being the limit in which one can give the remedy to bees. Cech (53) in a work published in 1877, also recommended carbolic acid

The Cheshire treatment (26) consists in using a treatment containing half a decilitre of carbolic acid in a litre of water, thoroughly shaking it up until the acid is entirely dissolved, and using half a decilitre of this in a litre of syrup. In this treatment it is also necessary to reduce the infected stock to the number of frames it can use, and if the queen is diseased to destroy her and substitute a healthy one. The syrup is given by pouring it into the empty cells of the brood nest.

This method of treatment has been frequently reported to be successful; but there have been many failures, perhaps partly owing to the fact that it is difficult to get the bees to take the medicated syrup

Experiments on the Antiseptic Value of Carbolic Acid According to McKenzie (28), two per cent. carbolic acid does not kill the spores in six days. One per five hundred of the acid prevented the germination of the spores, but when taken out of the solution and placed in ordinary beef broth it gave luxuriant growth. Hence McKenzie thinks that the explanation of the value of carbolated syrup in the treatment of foul brood consists in preventing the germination of the spores. The bee journals refer to numerous instances in which feeding carbolated syrup produced an improvement in diseased stock; but as soon as the treatment stopped, the disease broke out afresh.

Salycylic Acid. The salycylic acid treatment was first used by Hilbert in 1876. The following is the method of use:

Solution of Hilbert No 1—Pure salycylic acid, 12½ grams; alcohol, 100 grams.

Solution of Hilbert No. 2—200 drops of solution No. 1 (about five grams) in 200 grams of distilled water or rain water.

Fumigation—One or two grams of the pure acid for fumigation.

Syrup—From 200 to 240 drops of Solution No. 1 (or about 5 to 6 grams) in a litre of syrup, well mixed before the syrup cools.

As soon as the disease is noticed the hive is disinfected and the syrup fed; and this treatment is also used for other colonies as a preventive treatment. The fumigation is accomplished in a kind of tin lantern furnished with a small alcohol lamp, suspended over which is a small movable trough for placing the acid in. The flame of the lamp is regulated in such a manner that the acid is liquified and slowly evaporated without burning. Too great heat will decompose it and render it ineffective. The fumes of the acid spread through the hive in the form of a white vapour. Whilst the fumigation is in progress the entrance boards and all parts that can be disinfected are washed with No 2 solution. Fumigation and washing are repeated every 4 or 5 days until a cure is effected. The diseased colonies receive, every second evening, ⅛ of a litre of acid syrup; and it is wise to give the same treatment to the neighbouring hives. A cure is usually effected in 3 or 4 weeks. If later, it is generally regarded as a sign that the queen is diseased, in which case it would be well to replace her. Occasionally the queens die during the treatment; but this is not frequent.

This treatment was very successful in diseased hives belonging to Bertrand (59). All the hives that were treated, were cured. Cowan (60), who has also used Hilbert's treatment with some slight modifications, has had the same success; and such is his confidence in the treatment that he does not fear to introduce into his apiary foul brood colonies for treatment. Some have found the treatment ineffective; but Bertrand thinks (59) that in all such cases there has been something lacking in the work, some precautions overlooked or neglected.

Experiments on the antiseptic value of salycylic acid. Salycylic acid Agar was made containing 5 grams of 12½ per cent. solution of salycylic acid in one litre of agar. Petrie plates were made from this and streaked on the surface with *B. alvei.* At the same time control cultures on ordinary agar were made. The results were abundant growth on the control plates and good growth, (but somewhat less than on the control plates) on the salycylic acid agar.

Salycylic acid Vapour. One gram of the acid was evaporated in our laboratory according to the directions given by Bertrand (59), in a box about the same size as a hive. Agar plates streaked with spores of *B. alvei* were left in different parts of the box during the fumigation for 10 minutes. The plates were then taken out, the covers put on and the plates incubated at 37°C. for 48 hours.

Results. Fumigated plates—no growth.
Control plates—abundant growth.

From these experiments it will be seen that the vapour of salycylic acid acts antiseptically, and that the feeding of the acid in the syrup, in the proportions specified, probably acts as a stimulant to the bees, enabling them to withstand or throw off the disease.

(3) *Camphor.* Ossipow (61) was the first to use camphor as a curative; and Bertrand (59) describes the use of it as follows: "There is," says he, "placed upon the bottom board of the hive, enveloped in a piece of muslin, "a piece of camphor about the size of a walnut, which is replaced when it has "evaporated. The presence of the camphor permits the bees to clean out the "cells containing dead larvæ and stop the development of the disease. So "long as a hive contains some of the substance foul brood will not develop, 'at least according to our experience and to that of several other beekeepers. "The first thing to do then, when one doubts the state of health of a colony, "is to employ the Ossipow remedy before proceeding to more radical means. "One can administer camphor in food by dissolving it in its own weight of "alcohol."

Experiments on the antiseptic value of Camphor. Sloped agar in tubes was inoculated with one loopful of spores of *B. alvei*, and a crystal of camphor about the size of a large pea was dropped into the tube. The tubes were then capped with tin foil paper and kept at 22°C. and 37°C.; and control cultures were made at the same time. At 22°C., after two days, there was good growth in the camphor tube. At 37°C., after two days, compared with the control tube, the camphor tube showed slight restriction of growth, the extra heat having evaporated the camphor more quickly.

Another series was made by using agar Petri plates streaked with 2 loopfuls of spores. In each plate was placed a portion of camphor about the size of a large pea; and the plates were incubated at 37°C. In 24 hours there was good growth; but close to the lump of camphor, growth was slightly inhibited.

Thus, camphor in the quantity in which it might be kept in a hive has no antiseptic effect, the amount used in the experiments being far larger than would be used in a hive. This substance, therefore, if it has the effect mentioned by those who have used it, must act as a stimulant, strengthening the bees to overcome the disease.

(4) *Thyme.* Klempin (62) has used branches of dry thyme with success, burning them in the smoker for disinfecting his hives; but their effect, like that camphor, is not radical, and beekeepers are not all in accord as to their efficacy.

(5) In connection with thyme *thymol* may be mentioned. Zehetmayr (63) has recommended the use of thymol, and has made a little machine by which he steams the bees with this substance. If a little of it is placed in a hive it will prevent infection, because bees from uninfected hives will not come near it,—they object to the smell, until they become accustomed to it. Blow (63) thinks it very valuable, and Jones (65) remarks that, even in great dilution, it prevents the growth of the germ; but Cowan criticises its use, because it is disagreeable to bees, and if used in sufficient quantity, acts as a poison, and therefore cannot be good in food.

Experiments on the antiseptic value of Thymol. Crystals of thymol were placed in test tubes of sloped agar in our laboratory and inoculated with one loopful of spores of *B. alvei.* These were capped with tin foil paper and incubated at 22° and 37°C.

Result. Control tubes—abundant growth.
Thymol tube at 22°C.—slight growth.
" " 37°C.—very slight growth.

Agar plates, poured and streaked with two loopfuls of spores of *B. alvei*, were used in another experiment; and a piece of thymol the size of a large pea was placed in each plate. The plates were incubated at 37°, along with control plates, with the following results:

24 hours, control plate—abundant growth
" thymol plates—good growth, but close to the lump, no growth.

Hence we conclude that this substance has a very slight antiseptic effect.

(6) *Carbolic Acid and Tar.* These substances were first used by Schreuter (66) and they are applied as follows:—"A piece of felt wool is placed in a small box, and soaked with a mixture of carbolic acid and Norwegan tar, in equal proportions. The cover of the hive is slightly raised, in order to permit of the evaporation of the carbolic acid. The box is left upon the platform of the hive beneath the brood, and remains there permanently. The dose can be renewed as often as required. The addition of tar to the acid is for the purpose of making evaporation take place more slowly." This remedy has not been used to a very great extent. Borel (67) reports success with it; but others have not had the same results, and it is probable that it should be used only as a preventive.

Experiments on the antiseptic value of Carbolic Acid and Tar. Four drops of the mixture placed on blotting paper and inserted in a Petri dish containing agar streaked with spores, inhibited growth, from which we see that the mixture is antiseptic.

(7) *Creolin or Phenyle.* Creolin has been recommended by Cowan (68) and has been used with success by other apiculturists.

Recipes : Solution No 1—for sprinkling, disinfecting, etc.—half a teaspoonful of soluble creolin in a litre of water.

Solution No. 2 For washing hives, platforms, etc.—two teaspoonfuls of soluble creolin to a litre of water.

Solution No. 3—for feeding—a quarter of a teaspoonful of soluble creolin in a litre of syrup.

The water of the syrup ought always to be poured upon the top of the creolin and thoroughly mixed with it; and the mixture should be well shaken before using.

Use. Prepare a hive and a proper floor board, which has been washed with solution No. 2. Then, after having taken out the comb from the infected hive, shake off the bees, and sprinkle the comb with solution No. 1. Take out all superfluous comb and spray it with solution No. 2, and extract the honey from it. The honey can then be boiled, and if it is used for feeding the bees, it can be diluted and phenol added in the proportion of one quarter to a teaspoonful to a litre of the diluted honey. The combs are then put back and the bees fed with medicated syrup. If the bees take the syrup, the dose can be gradually increased; but we must be careful not to give more than one teaspoonful to a litre of syrup. If the bees refuse to touch it, which is not at all improbable, if they have access to other food, pour the medicated syrup upon the neighboring combs, when the bees will quickly become habituated to it, and afterwards will take it in the ordinary manner. The vapour of creolin also acts as a disinfectant. A small phial of concentrated creolin may be placed in a corner of the hive, and lightly stopped with a cotton plug; and the lower part of the cotton being in contact with the liquid, capillarity will take place and draw up the creolin, and the heat of the hive will produce the necessary evaporation. A piece of blotting paper can be used by saturating it with creolin, and placing it upon the floor board or in a box covered with perforated zinc, so that the bees will not come into contact with the disinfectant.

Creolin is neither poisonous nor corrosive for man ; but, in strong doses, it kills insects. Consequently it is necessary not to give greater strengths than those mentioned above. In the use of this remedy it is necessary to stimulate the production of brood by feeding liberally with medicated syrup; if the disease does not yield to this treatment. the queen should be removed.

Experiments on the antiseptic value of creolin. a. Sloped agar—each tube, inoculated with one loopful of spores, was plugged with cotton wool, saturated with creolin, and then capped with lead foil Tubes were kept at 22° C. and 37° C.

Result : After four days at 22° C.—No growth, except beneath the condensation water in the tubes.

After four days at 37° C.—No growth.

At the end of this time new cotton plugs were inserted into the tubes in the place of the creolin ones, and the cultures again incubated, when good growth ensued in 24 hours.

b. Agar plates were made and streaked with two loopfuls of spores. In each plate was placed a square inch of thick blotting paper, with four drops of creolin on it. The plates were kept in the incubator at 37° C., and removed in 48 hours, when very slight growth was manifest. On removal of the creolin and further incubation of the plates, good growth was obtained. Control plates gave copious growth. These experiments were repeated with only one drop of creolin.

Result, after 24 hours—abundant growth. With two drops of creolin, the growth was restricted to the inoculation track after 48 hours at 37° C.

c. In addition to the above experiments, agar was made containing the same proportion of disinfectant as was used in feeding the bees of diseased hives; 15 c. c. of this agar was taken for making a plate culture, and several plates were streaked with two loopfuls of spores, and incubated at 37° C. Strength of agar,—2 c. c. creolin to 1 litre of water, i.e., about half a teaspoonful to a quart.

Results —Creolin agar, four tests—no growth.
Control agar, abundant growth.

This antiseptic in the strength used by Cowan for feeding purposes, would prevent the germination of the spores; and if there was a large amount evaporating in the hive, a slight antiseptic result would take place.

(8) *Eucalyptus* This substance was introduced by Beauverd (69). A small tin box, with a cover pierced with small holes, is placed upon the floor board of the diseased hive, and filled with essence of Eucalyptus. The colony receives every four or five days a litre of syrup containing a teaspoonful of tincture of eucalyptus (oil eucalyptus, 1; alcohol, 9). Then from time to time some drops of the same tincture are dropped into the hive. Auberson, who was the metayer of Bertrand's Apiary and was managing his own higher up the mountains, cured a number of colonies by means of this method. He finds that there is a great difference in the effect produced by the remedy. In some cases, the effect follows the remedy quickly; in others, the effect is slower. Sometimes more than a year passes without resulting in a complete cure. When the disease is of long standing, the remedy must be proportionate to the gravity of the evil. When there are only a few diseased cells, Auberson simply pours some drops of the essence along the back wall of the hive. He renews the dose every eight days; and in six weeks, sometimes sooner, the colony is cured. In cases where the hive is badly affected, he takes a clean hive and floor board and impregnates the interior, floor board, and division board with eucalyptus, and then transfers combs, brood, and bees to the new hive. He leaves the foul brood colonies their rotten combs, as this is the only handy means of disinfecting them. Three weeks later, during which he has twice poured eucalyptus on the floor board, he examines the new brood. If it exists in healthy patches he simply pours a few drops of the essence on the floor board until the cure is complete. If, however, the fresh brood still disclose some diseased spots, the queen is killed and replaced by another, and every fifteen days the essence is spread on the floor board until the cure is completed. If the colony is very weak, he strengthens it by the addition of bees and healthy brood. If he has to feed a diseased hive, he never fails to put the essence in the syrup.

Besides these well authenticated cases of cure by the essence of eucalyptus, there are a number of others, and the method has been extensively used in Europe. The great drawback to the use of this remedy is that it is liable to cause robbing.

Experiments on the antiseptic value of eucalyptus. (*a*) Eucalyptus oil. The cotton plug of a spore-inoculated sloped agar tube was saturated with the oil, and incubated at 37° C. In eighty-four hours there was no growth, but a fresh plug being inserted good growth occurred in twenty-four hours.

(*b*) Agar plates inoculated with spores and containing four drops of eucalyptus on a piece of blotting paper were incubated at 37° C. No growth formed, but when the eucalyptus was removed good growth immediately ensued. On plates containing two drops the growth was restricted to the inoculation track, but when the oil was removed abundant growth took place. On plates containing one drop on blotting paper there was abundant growth in twenty-four hours.

(*c*) Eucalyptus agar was made by using a teaspoonful (4 c.c.) of tincture of eucalyptus to a litre of agar. Six plates were made with eucalyptus agar, each plate inoculated with spores, with the result that the growth on the medicated agar was only slightly less that that on the control agar. The medicated agar smelt slightly, but characteristically of eucalyptus oil.

A Queensland (Australia) correspondent of the *British Bee Journal* (71) is of the opinion that no foul brood exists among bees in that country. The reason of this is that the honey that goes into the combs is largely gathered from the eucalyptus, the medicinal qualities of which combat foulness in all forms. This statement, however, is not reliable, inasmuch as foul brood is known to be prevalent in Queensland.

(9) *Napthol Beta.* Napthol Beta was first used as a remedy by Lortet (72). The treatment is as follows:

The drug is administered in the food, in the proportion of one-third of a gram to a litre. This one third of a gram is at first dissolved in a little alcohol, as it is extremely insoluble in water. Afterwards it is mixed in a litre of water, and this liquid is used for making the syrup. In England the usage is to dissolve the napthol in the sugar, the proportion being about forty to fifty centigrammes to a kilo of sugar. It is, however, better to dissolve it in alcohol. Lortet thinks that external treatment by means of fumigation or spraying is helpful, as these methods contribute largely to the disinfection of hives, comb, etc.; but as he believes that it is always the digestive canal of the nurse bee which is infected and that it is by the act of feeding that the adult bee infects the digestive canal of the larvæ, therefore all efforts should be directed to the digestive canal of the worker bees, and the treatment ought to be internal and as energetic as possible. He states that when administered in the proportion of 0.33 gram per 1,000 of liquid it prevents all fermentation and decomposition and other changes caused by microbes. He further maintains that in addition to the use of this preparation first-rate hygienic conditions are necessary in order to give the bees vitality and recuperative power, which play an important part in enabling living organisms to resist the inroads of virulent microbes.

McKenzie found that (28) a beef broth containing one per thousand of B. Napthol prevented spores of *B. alvei* from germinating, and consequently had an equal value with one per five hundred of carbolic acid.

This remedy has been widely used and with considerable success.

Experiments on the antiseptic value of Napthol Beta. Napthol Beta agar was made in our laboratory the same strength as that recommended by Lortet for feeding, that is 0.33 gram B. Napthol to one litre of agar. Eight tests were made in Petri dishes, inoculated with spores of *B. alvei*, and in no case did growth result; from which we learn that a dilution of one-third of the solution used by McKenzie completely inhibited growth. Napthol Beta agar containing 0.165 gram of the drug to a litre of agar was also tried, and the result of a number of tests was that some growth took place on the medicated plates and abundant growth on the control plates.

From these experiments, also those of Lortet and McKenzie, it will be seen that Napthol Beta has a strong antiseptic action.

(10) *Naphthaline.* This substance is regarded as a preventive rather than as a curative, although there are cases known in which it has effected a cure of diseased hives. A small quantity of the drug is placed on the floor board of the hive, a crystal about 2 c m. in diameter as far from the entrance

of the hive as possible. The evaporation is rapid and with very strong odour. Hence, if too much used, the brood will be deserted by the workers and death of the bees may take place. As soon as the dose has evaporated it is renewed.

As a preventive, naphthaline has been very favourably reported upon by a number of writers; and Cowan (73) states that he inspected very thoroughly a hive belonging to Merney which had been cured by this substance.

Experiments. In our laboratory, crystals of naphthaline about the size of a large pea were put into test tubes containing sloped agar, inoculated with one loopful of spores, capped with tin foil paper and kept at 22° and 37° C.

Results. After 48 hours—good growth in all tubes. Inoculated agar plates containing a crystal of naphthaline likewise gave good growth in 24 hours at 37° C, as did also the control tubes and plates. Hence, we conclude that naphthaline has no antiseptic power; and we are forced to look upon its use rather doubtfully. It may, perhaps, act as a stimulant.

(11) *Formic acid.* This substance was first suggested by Dennler in 1885 (74), but he did not ascertain the strength in which it could be used. Sproule (75) states, that since the year 1882 he had successfully treated foul brood with formic acid. He was the first apiculturist to use the remedy and give the treatment. The solution used is pure acid, 10 parts; water, 90 parts; and the treatment is as follows:—

A part of the comb is taken from the hive and as many bees as possible are shaken from the diseased comb; and then two or three empty combs are used, into one of the sides of which 100 grams of the solution are poured, while it is held inclined so as to allow the liquid to run into the cells and stay there. These combs are placed on each side of the brood, the side containing the solution next the brood. Eight or ten days after, an inspection is made; and if there is no cure, the dose is renewed and continued every week until the cure is complete, which is often after the first treatment. In fact the disease rarely resists the second or third application. To hasten the cure, this remedy can be given in the food of the bees—a teaspoonful to a litre of syrup.

Experiments Formic acid probably has an important rôle to play in the keeping properties of honey. As long ago as 1878, formic acid was found in honey; and Muhlenhoff (76) observed that when honey is not intended for immediate use, the bee deposits in each cell a drop of formic acid, secreted by the venom glands, and then seals the cell. Erlenmeyer (77) says that formic acid of the strength of 1.205 gr. to a thousand parts of water was antiseptic, Planta (78) refutes Muhlenhoff's idea that 100 grams of sealed honey contains .0186 grams of 22% formic acid. "100 grams is the capacity of 165 worker cells, but the smallest droplet of venom contains at least .0254 grams of formic acid, which would make for 165 cells, 4 1910 grams; that is to say, 200 times more than there is in reality." This opinion is, however, contrary to one expressed before by the same writer, in the year 1884 (79).

Formic acid seems to help bees to ward off the disease, especially when we supply it to them ready made; and that found in certain kinds of honey has probably an antiseptic effect. Two samples of clover honey and two samples of buckwheat honey were analyzed in our chemical laboratory with the following results:—

1	Buckwheat honey	0 15	grains of formic acid in 100 grains of honey.
2	"	0.17	" " " "
1	Clover honey	0 0579	" " " "
2	"	0.057	" " " "

Formic acid agar was then made containing the same proportion of formic acid as was found in the first sample of buckwheat honey, and weaker formic acid agar containing the same percentage of formic acid as was present in the first sample of clover honey; and spores placed upon the stronger formic agar did not germinate, while on the weaker formic agar the germination was only slightly retarded; and after the weaker agar was two days in the incubator, there was a large growth. Spores transferred from the strong formic agar (after being in contact with it for six days in the incubator) failed to grow on the weaker formic agar within two days; but after four days in the incubator they grew abundantly. The culture growing on the weaker formic agar was then transferred to the strong formic agar, to ascertain whether the germ could be accustomed to more unnatural food by previous cultivation on the weak formic agar. This transfer was, however, unsuccessful.

The germs used in these tests were isolated from samples of diseased comb from Ontario, Austria and Florida, U.S.A.

Formic acid bouillon was also made containing .15% of formic acid; and spores kept in this broth for eight months continued to germinate when transplanted to suitable material.

Formic acid agar was likewise made in the same proportion as suggested by Bertrand (59); that is, formic acid 10, water 90; and a tablespoonful of this mixture to a litre of syrup; but instead of syrup, agar was used. Fifteen c.c. of this acid agar was poured into each Petri plate, and the surface inoculated with spores.

Results: On 14 plates, no growth.
On 2 plates, very restricted growth, limited to one-eighth of an inch of the needle track (60 hours).
On control plates, abundant growth.

From these investigations, viz., the analysis of the honey, the experiments based thereon, and the tests with agar made in the proportion suggested by Bertrand, we would note three things: (1) That the amount of formic acid recommended by Bertrand for the cure of foul brood is almost identical with the amount found in buckwheat honey; (2) that formic acid is a good antiseptic; (3) that the formic acid in buckwheat honey may possibly tend more or less to ward off foul brood.

We may add that our analysis, showing a larger proportion of formic acid in buckwheat honey than in clover honey, is an interesting explanation of a fact well known among practical bee-keepers, viz., that the sting of bees when working on buckwheat is much more irritant than when working on clover.

In conclusion under this head, we may say that formic acid has given good results when used in the treatment of foul brood; and it is in a sense a natural remedy, being manufactured to some extent by the bees themselves.

(12) *Other substances used for treating this disease.* Among other substances that have been used for treating this disease are sulphuric acid, sulfaminol, various modifications of substances already mentioned, and some recommended in the McLean method (80), the Muth method, and others; but these have not had so wide application as those referred to in the preceding paragraphs.

EXPERIMENTS ON THE USE OF DRUGS FOR COMBATTING THE DISEASE.

I have already mentioned that, in one of my experiments, I endeavoured to find out if the virulence of the germ was attenuated by prolonged culture in artificial media, with the result that considerable attenuation occurred after a large number of transfers; and in the following experiments I have endeavoured to meet any objections that might be made as to the virulence of my cultures, by isolating *B. alvei* from a badly diseased hive and then growing at once sufficient spores for the purposes of the experiment. Thus but three transfers from a diseased larva were made; and all the spores used in the following experiments were obtained in this manner:

Two small hives, each containing strong healthy swarms, were selected and placed side by side.

Hive A was given spores of *B. alvei* in syrup containing one-third of a gramme of napthol B. to a litre of syrup

Hive B was given spores of *B. alvei* in syrup containing from 1.6 to 1.8 c c. formic acid to a litre of syrup.

The spores given were scraped from the surface of an agar slope culture, put into 10 c c. of sterile water, and well shaken in order to obtain a good suspension of spores. The water and spores were poured into medicated syrup and the mixture thoroughly stirred. It was then given to the bees and was readily accepted. This procedure was continued four days a week for three weeks, and at the end of this time each hive had received the whole of the growth from twelve sloped agar tubes. During the feeding period the combs containing the brood were carefully examined, but none of the usual symptoms of the disease appeared, although cultures were obtained from different parts of the hives and from the digestive tract of the workers. At the end of three weeks the medicated syrup was discontinued for a week. Then ordinary syrup containing spores was given, and at the end of ten days typical symptoms began to be noticed, and after sixteen days the disease was well establshed Both hives, so far as I was able to judge, were the same—no disease to be seen in either whilst medicated syrup was fed, but infection manifest in both soon after the formic acid and napthol B. were discontinued. This experiment goes to prove the benefit of feeding with syrup a substance which is antiseptic and which hinders the germination of the spores. It also confirms Lortet's opinion that the digestive canal of the nurse bee is alone *infected*. I have never been able to obtain Cheshire's results, viz, the isolation of the bacillus from the blood of the worker, but I have frequently found it in the digestive canal of bees from diseased colonies.

From the results of the above experiments I conclude that in certain cases the use of chemicals is beneficial, but I would not say that other measures, such as starvation and stamping out, should be abandoned as unnecessary or useless. Some of the drugs used are of very little, if any, value; but others, such as formic acid and napthol B, are undoubtedly very useful. In some cases, especially those in which the disease is very virulent, it may be advisable to resort to more drastic measures.

TOXINS.

I endeavoured to find out whether or not the feeding of toxin (filtrate from a two weeks old culture of *B. alvei* in saccharose bouillon) mixed in syrup would enable healthy bees to withstand the disease. Small amounts of this filtrate were given in syrup to a healthy colony every other day for three weeks. The amount of filtrate fed was gradually increased, but as the amount got larger the bees refused to take it, so it had to be poured over the combs. At the end of three weeks spores of *B. alvei*, freshly isolated, were fed, and symptoms of the disease followed about fourteen days later. So the toxin had little or no effect, but further experiments are being made.

LEGISLATION.

In the United States, six States have laws for the suppression of foul brood among bees. These are New York, Wisconsin, Michigan, Utah, Colorado and California. In Canada the Province of Ontario has enacted a foul-brood law. In Europe Mecklenburg also has a law.

These statutes differ a good deal from one another, and some of them are so drafted that evasion of the law is easy. The best are probably those of Wisconsin and Ontario, and the principal points in these acts are as follows:

1. The appointment of an inspector.
2. The inspection of all apiaries reported as diseased, and the duty of the inspector, if satisfied that the disease is present, to give full instructions as to treatment.
3. The enactment requiring the inspector, who is the sole judge, to make a second visit to all diseased apiaries, and, if need be, burn all colonies and combs that he may find uncured.
4. Various penalties (fines, and, in default, imprisonment) for—
    (*a*) Selling or giving away diseased colonies or infected appliances.
    (*b*) Selling bees after treatment, or exposing infected appliances.
    (*c*) Obstructing the inspector.
5. Persons who are aware of the disease either in their own apiary or elsewhere are to notfiy at once the proper authorities, and in default of so doing shall, on conviction, be liable to a fine and costs.
6. The inspector of apiaries to make an annual report, which shall include a statement of the number of colonies destroyed by his order, the localities where found, and the amount paid to him for his services.

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